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RESEARCH ARTICLE

A new cecidogenous species of many-plumed moth (Alucitidae) associated with *Cordiera* A. Rich. ex DC. (Rubiaceae) in the Brazilian Cerrado

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ABSTRACT. Larvae of many-plumed moths (Alucitidae), especially in the world-wide genus *Alucita* Linnaeus, 1758 are known as borers or gall-inducers on flowers, fruits and shoots of a few dicotyledonous families, including Bignoniaceae, Caprifoliaceae and Rubiaceae. However, there is no study available on the biology of the monotypic, Neotropical genus *Prymnotomis* Meyrick, 1931 except for its original description that was based on a single male, the holotype of *Prymnotomis crypsicroca* Meyrick, 1931 from Espirito Santo, Brazil. We describe here a second species for this genus, *Prymnotomis cecidicola* **sp. nov.** whose larvae induce galls on *Cordiera elliptica* (Cham.) Kuntze (Rubiaceae), a dioecious plant with dimorphic inflorescences found in the Brazilian Cerrado, Planaltina City, Federal District. Adults, larvae, pupae and galls are illustrated under light and scanning electron microscopy. Galls are green, spherical, unilocular and develop individually on *C. elliptica* flower buds. During development they look like fruits in shape and colour but are larger, do not have style scars when on female plants, and are induced also in male inflorescences. Pupation occurs outside the gall within a silk cocoon, presumably in the litter. A preliminary analysis of DNA barcode sequences including putative members of other alucitid lineages and Neotropical BINs (Barcode Index Number) supports *Prymnotomis cecidicola* **sp. nov.** as an independent phylogenetic unit, with 12 to 18% divergence. Its nearest-neighbour was the BIN cluster 5 (BOLD:AAA0842) that includes specimens from Costa Rica.

KEY WORDS. Alucitid moths, Brazilian Savanna, insect galls, *Prymnotomis*, taxonomy.

INTRODUCTION

Alucitidae is a small, worldwide family of apoditrysian moths with nine valid genera and ca 216 species (Gielis 2003, Nieukerken et al. 2011). Several additional alucitid species have been described for the family recently, particularly in the tropics (e.g., Vargas 2011, Heppner 2015, Ustjuzhanin and Kovtunovich 2016, Ustjuzhanin et al. 2018), and many are supposed yet to be discovered in these regions. A total of 27 species of alucitids are found in the Neotropics, of which only six have been recorded in Brazil (Gielis 2003, Vargas 2011, Heppner 2015). These micromoths are well known by their specialized fore- and hindwings, which are multiply divided into lobes that look like bird feathers. Larvae are usually borers or gall-inducers, associated with flowers, fruits and shoots of a few plant families including the Bignoniaceae, Caprifoliaceae and Rubiaceae (Dugdale et al. 1998). In contrast to the adults, their immature stages are poorly known, especially in the Neotropical region where the host plants for a few species have been documented, particularly within *Alucita* Linnaeus, 1758 the most speciose, worldwide genus (e.g. Vargas 2011). Four other endemic genera have been described for the region: *Hexeretmis* Meyrick, 1929, *Prymnotomis* Meyrick, 1931, *Paelia* Walker, 1866, and *Alinguata* Fleming, 1948; to the best of our

knowledge, none of the host plants or the immature stages of these genera have been identified so far (see also Lima 1945 and Pastrana 2004). Taxonomy of *Hexeretmis* and *Alinguata* was revised by Heppner (2010a,b), based on the adults.

Prymnotomis was proposed by Meyrick (1931) to include *P. crypsicroca* Meyrick, that was described briefly based on the general morphology of only one adult male from Espírito Santo, Brazil. It has remained monotypic genus since its original description, and there is no additional information about its biology. This study concerns a second, new species of *Prymnotomis* that induces conspicuous, spherical galls on inflorescences of *Cordiera elliptica* (Cham.) Kuntze (Rubiaceae), a native shrub of the Brazilian Savanna (Cerrado Biome). A preliminary comparison of genitalia structures suggested that it is congeneric with but is not *P. crypsicroca*. In this study we describe and illustrate the adult, larva, pupa and the gall under light and scanning electron microscopy, and provide information on the natural history of this new species. An analysis of DNA barcode sequences including putative members of other alucitid lineages, as well as Neotropical BINs (Barcode Index Number; Ratnasingham and Hebert 2013) is also provided to estimate the phylogenetic position and genetic distances of the new species.

MATERIAL AND METHODS

Adult specimens were reared by V.O. Becker from galls collected during October of 1981, 1982, and 1983 at the Centro Nacional de Pesquisa Agropecuária dos Cerrados (Embrapa Cerrados), Planaltina City, Federal District, Brazil (15°36'26.4"S; 47°42'52.4"W), and maintained in small plastic pots under room temperature in the Laboratório de Entomologia of the same Institution. They were checked daily for the emergence of adults, which were pin-mounted and dried. Immatures used for descriptions were dissected from additional galls that were collected by C.M. Pereira and A. Specht at same locality during September 2018, and brought to the Laboratório de Morfologia e Comportamento de Insetos (LMCI), Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil, where they were reared in a similar manner. They were fixed in Dietrich's fluid and preserved in 75% ethanol. Additional larvae used for DNA extraction were preserved in100% ethanol at -20 °C.

For descriptions of adults, genitalia were dissected and cleared in a 10% potassium hydroxide (KOH) solution, stained with either eosin or Chlorazol black E and slide-mounted in Canada balsam, following Robinson (1976). Last instar larvae were prepared similarly for the study of chaetotaxy. Observations were performed with the aid of a Leica® M125 stereomicroscope. Structures selected to be drawn were previously photographed with a Sony® Cyber-shot DSC-H10 digital camera attached to the stereomicroscope. Vectorized line drawings were then made with the software Corel Photo-Paint® X7, using the corresponding digitalized images as a guide.

Additional specimens were used for scanning electron microscope analyses. They were dehydrated in a Bal-tec® CPD030 critical-point dryer, mounted with double-sided tape on metal stubs, coated with gold in a Bal-tec® SCD050 sputter coater and examined and photographed in a JEOL® JSM6060 scanning electron microscope at the Centro de Microscopia e Microanálise (CMM) of UFRGS.

Terminology used in descriptions followed Heppner (1987), Patočka and Turčani (2005) and Landry and Landry (2004) for the larva, pupa and adults.

DNA was extracted from larvae of four specimens (CMP 008-01A, D) of *Prymnotomis* sp. nov. using the PureLink Genomic DNA extraction kit (Thermo Fisher Scientific, Carlsbad, California). Extracted DNA was resuspended in 80 mL of Tris: EDTA (10 mm Tris-HCl, 1 mm EDTA, pH 5 8.0). DNA barcoding PCR was conducted using primers LCO1490 and HCO2198 (Folmer et al. 1994), which amplify ca. 500 bp of the mitochondrial gene cytochrome oxidase (COI). PCR reactions were conducted using 2 µL of the extracted DNA. The thermal cycler profile consisted of 35 cycles of 94 °C for 45 s, 48 °C for 45 s and 72 °C for 45 s. Excess dNTP and primers were removed and the amplified DNA concentrated using exonuclease I and FastAP thermosensitive alkaline phosphatase (Thermo Fisher Scientific). Samples were sequenced in both directions using BigDye Terminator v. 3.1 Cycle Sequencing kit (Thermo Fisher Scientific) following standard procedure according to manufacturer's instructions, and analysed in an ABI3730XL (Thermo Fisher Scientific, Waltham, USA) automatic sequencer. The new data were deposited in BOLD Systems (http://www.boldsystems.org/) under the project MISA. The barcode sequences were aligned using CodonCode Aligner (CodonCode Corp, Massachusetts, USA).

To explore the phylogenetic position of the new species within the family we used the COI data generated for *Prymnotomis cecidicola* sp. nov. with a published dataset of 40 Alucitidae (Table 1). Specifically, the genera *Alucita* and *Pterotopteryx* Hannemann, 1959 were included in the analysis, as well as five BIN clusters (BOLD: AAH5751, AAG9907, AAJ6491, AAU0280 and AAA0842) that were identified in the Neotropics, publicly available in BOLD (Table 1). The tree was rooted with *Isonomeutis amauropa* Meyrick, 1888 (Copromorphidae) according to the phylogeny proposed by Mutanen et al. (2010). We used the maximum likelihood algorithm, which was performed in PHYML v. 3.0 (Guindon et al. 2010) using 1000 replicates of heuristic search, with random addition of sequences and TBR branch swapping. The Tamura-Nei substitution model was selected based on the Akaike information criterion run in MEGA v. 6 (Tamura et al. 2013). Monophyly confidence limits were assessed with the bootstrap method at a 50% cut-off after 1000 iterations. Pairwise genetic distances between the new species and other Alucitidae were quantified using the Kimura 2-parameter model in MEGA v. 6.

Museum collections. AMNH – American Museum of Natural History, New York, NY, USA; DZUP – Coll. Padre Jesus S. Moure, Departamento de Zoologia, Universidade Federal do

Table 1. Sample information for specimens used in this study.

Species	Acession number			
	Sample ID	Genbank	BOLD COI-5P	BIN clusters
Ingroup			–	–
Alucita adriendenisi	CGWC-3887	–	LOWCE127-06	–
Alucita cancellata	TLMF Lep 03910	JN307745	PHLSA260-11	–
Alucita debilella	TLMF Lep 03912	JN307747	PHLSA262-11	–
Alucita desmodactyla	TLMF Lep 09154	KP253214	PHLAI592-13	–
Alucita grammodactyla	JBA-05-0004	–	LTOL071-06	–
Alucita hexadactyla	TLMF Lep 03909	JN307744	PHLSA259-11	–
Alucita lalannei	jflandry2557	–	MECC537-06	–
Alucita montana	BIOUG21945-E01	–	SMTPL7338-15	–
Prymnotomis cecidicola sp. nov.	CMP 008-04A	MK919497	MISA041-19	–
Prymnotomis cecidicola sp. nov.	CMP 008-04D	MK919498	MISA042-19	–
Pterotopteryx dodecadactyla	TLMF Lep 08735	KM573355	PHLAH931-12	–
alucitBioLep01	BioLep698	HQ936333	BLPDT350-10	BOLD:AAA0842
alucitBioLep01	BioLep698	HM411224	BLPDM2386-10	BOLD:AAA0842
alucitBioLep01	BioLep698	HQ555946	BLPDQ816-10	BOLD:AAA0842
alucitBioLep01	BioLep698	HM402693	BLPDN1595-10	BOLD:AAA0842
alucitBioLep01	BioLep698	HM402110	BLPDN1038-10	BOLD:AAA0842
alucitBioLep01	BioLep698	HQ555644	BLPDQ346-10	BOLD:AAA0842
Alucita	BioLep698	–	LTOL788-07|	BOLD:AAA0842
Lepidoptera	BioLep698	–	BLPDW575-11	BOLD:AAA0842
alucitBioLep01	BioLep698	HM403333	BLPDN2246-10	BOLD:AAA0842
alucitBioLep01	BioLep696	JN296976	BLPEA475-11	BOLD:AAU0280
alucitBioLep01	BioLep696	JN295546	BLPDX574-11	BOLD:AAU0280
Lepidoptera	–	–	NOUD2153-12	BOLD:AAG9907
Lepidoptera	–	–	BLPEF6714-14	BOLD:AAG9907
Lepidoptera	–	–	BLPEF6357-14	BOLD:AAG9907
alucitBioLep01	BioLep693	HQ934518	BLPDR306-10	BOLD:AAG9907
Lepidoptera	–	–	MHMYS2880-13	BOLD:AAG9907
alucitBioLep01	BioLep693	HM375200	BLPDF869-09	BOLD:AAG9907
Alucitidae	–	–	LMEMB642-09	BOLD:AAG9907
Lepidoptera	–	–	BLPEE4426-14	BOLD:AAH5751
Lepidoptera	–	–	BLPEE3748-14	BOLD:AAH5751
Lepidoptera	–	–	BLPEE3457-14	BOLD:AAH5751
Lepidoptera	–	–	BLPEE4540-14	BOLD:AAH5751
alucitBioLep01	BioLep694	–	BLPDY391-11	BOLD:AAH5751
alucitBioLep01	BioLep694	–	BLPDL1914-10	BOLD:AAH5751
alucitBioLep01	BioLep694	–	BLPDY535-11	BOLD:AAH5751
Lepidoptera	–	–	BLPEE3749-14	BOLD:AAH5751
Lepidoptera	–	–	BLPEE4316-14	BOLD:AAH5751
alucitBioLep01	BioLep694	HQ934494	BLPDR282-10	BOLD:AAH5751
Lepidoptera	–	–	BLPEE3667-14	BOLD:AAH5751
Lepidoptera	–	–	BLPEE3751-14	BOLD:AAH5751
Lepidoptera	–	–	–	–
	–	–	BLPEE3747-14	BOLD:AAH5751
Outgroup	–	–	–	–
Isonomeutis amauropa	MM11203	GU828850	LEFIA1190-10	–

Paraná, Curitiba, PR, Brazil; LMCI – Coll. Laboratório de Morfologia e Comportamento de Insetos, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; NM – Natural History Museum, Vienna, Austria; NHMUK – Natural History Museum, London, United Kingdom; VOB – Coll. Vitor O. Becker, Reserva Serra Bonita, Camacan, BA, Brazil.

RESULTS

Molecular data

Sequencing of COI resulted in an average amplicon size of 500 bp. The aligned data matrix had 683 characters, of which 227 (33%) were phylogenetically informative. Maximum likelihood analysis recovered an optimal ML ln likelihood tree = 5937 with nucleotide frequencies of A = 31.9%, C = 15.2%, G = 14.6%, and T = 38.3%. In the preliminary barcode tree all Neotropical specimens clustered, including *Prymnotomis cecidicola* sp. nov. and the five BIN taxa. Specimens of *Alucita* and *Pterotopteryx* grouped in a second clade, the Nearctic + Palearctic (Fig. 1). The nearest neighbour of *P. cecidicola* was the BIN cluster 5 BOLD:AAA0842 (ca 12% genetic distance), which included specimens from Costa Rica (data from BOLD). Pairwise genetic distance of the new taxon to BIN clusters and lineages within Alucitidae ranged from 12 to 18%, with the highest divergence to *Pterotopteryx dodecadactyla* (Hübner, [1813]) (Table 2).

Table 2. Genetic distance between *Prymnotomis cecidicola* sp. nov. and members of Alucitidae based on 683 base pairs of the DNA barcode sequences using the Kimura 2-parameter model. BIN clusters are identified in Fig. 1.

		1	2	3	4	5	6	7	8
1	*Prymnotomis cecidicola* sp. nov.	–							
2	*Alucita* spp.	0.16	–						
3	BIN cluster1 (BOLD:AAH5751)	0.16	0.17	–					
4	BIN cluster2 (BOLD:AAG9907)	0.14	0.15	0.08	–				
5	BIN cluster3 (BOLD:AAJ6491)	0.14	0.14	0.09	0.03	–			
6	BIN cluster4 (BOLD:AAU0280)	0.17	0.16	0.15	0.12	0.12	–		
7	BIN cluster5 (BOLD:AAA0842)	0.12	0.15	0.17	0.13	0.14	0.14	–	
8	*Pterotopteryx dodecadactyla*	0.18	0.14	0.17	0.15	0.15	0.17	0.13	–

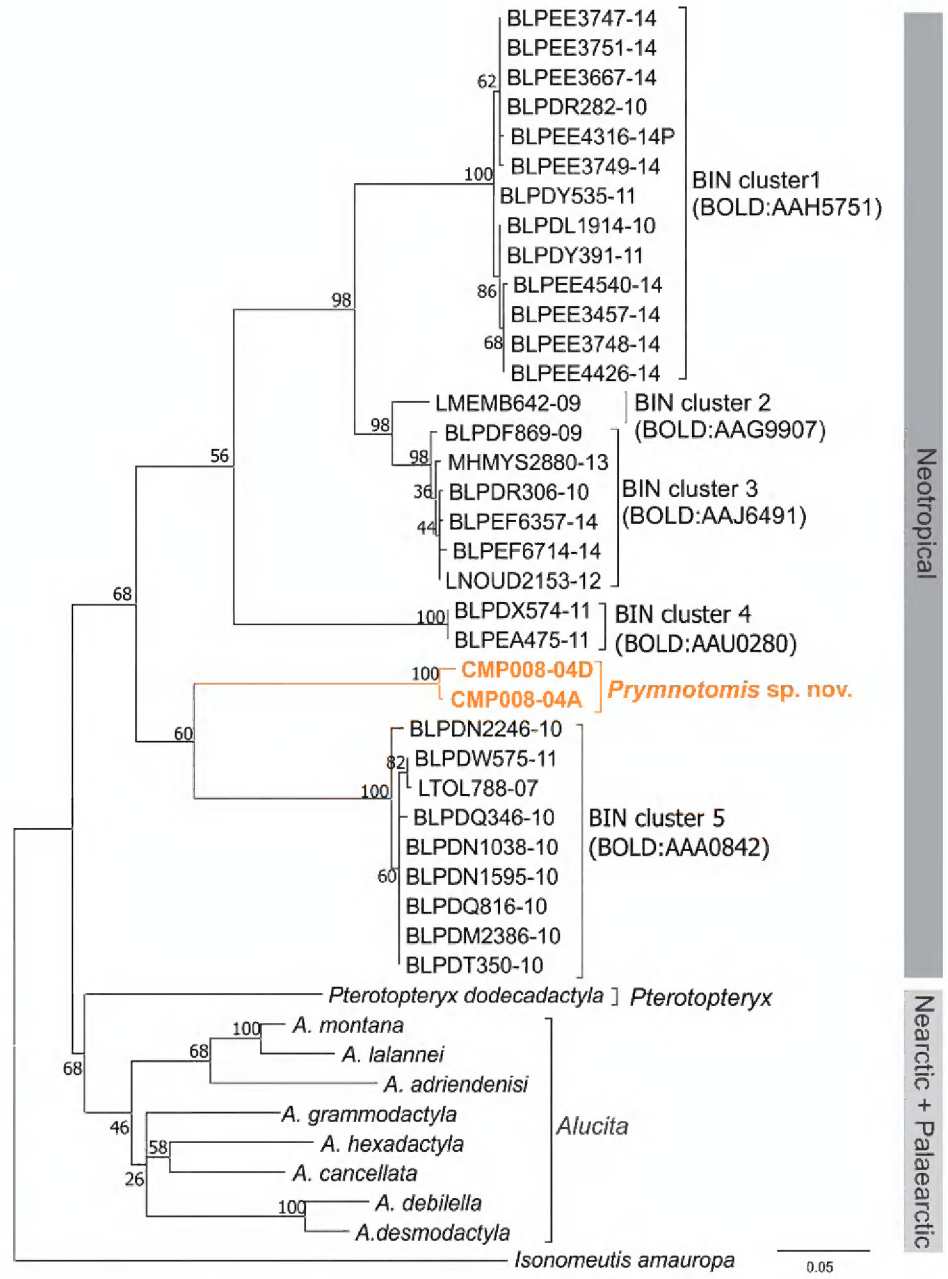


Figure 1. Maximum likelihood tree based on COI sequences for 41 Alucitidae species and lineages – ln likelihood = 5937. The phylogenetic position of *Prymnotomis cecidicola* sp. nov. (CMP 008) is indicated in orange. Bootstrap values are indicated for nodes with more than 50% support (1000 replications).

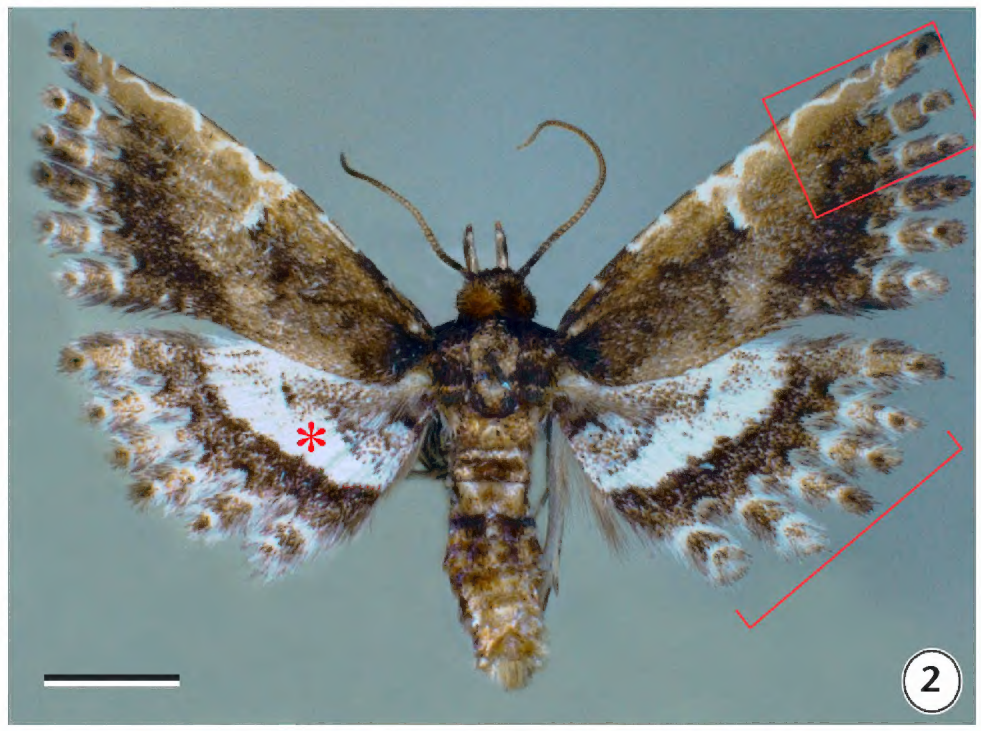
Taxonomy

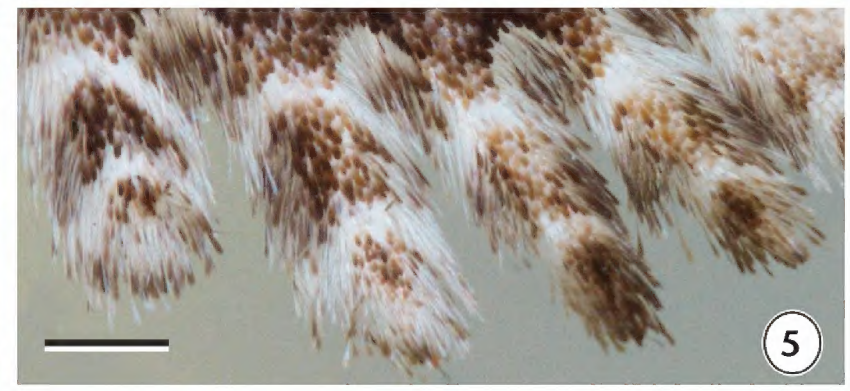
Prymnotomis cecidicola Moreira & Becker, sp. nov.

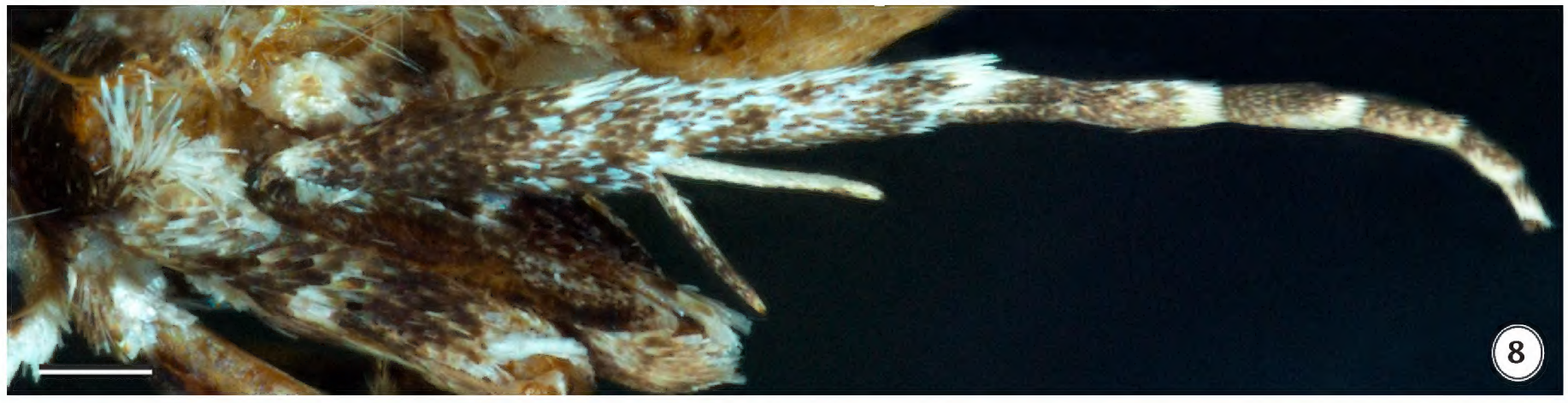
http://zoobank.org/bbb13a6b-e459-4e5b-bdab-c66c24270f68
Figs 2–48, 51, 54

Diagnosis. *Prymnotomis cecidicola* shares with the closely related *Hexeretmis* Meyrick the porrect maxillary palpi, forewing cleft only to 1/5 from termen, and pattern of wing venation. However, it differs from this genus in general appearance in coloration pattern and by presenting deeper hindwing clefts (ca 1/3 of wing length), as pointed out by Meyrick (1931). *P. cecidicola* can be separated from the congeneric *P. crypsicroca* Meyrick by the smaller size of the latter, whose forewing length of the type specimen measures ca 6 mm, and by the less contrasting coloration of *P. crypsicroca*, especially in relation to the hindwing. The basal process of the valva of the male genitalia is upturned, finger-like, covered with sparse filiform setae in *P. crypsicroca*, while in *P. cecidicola* it is turned down, looking like a claw, bearing short, stout spines on the base.

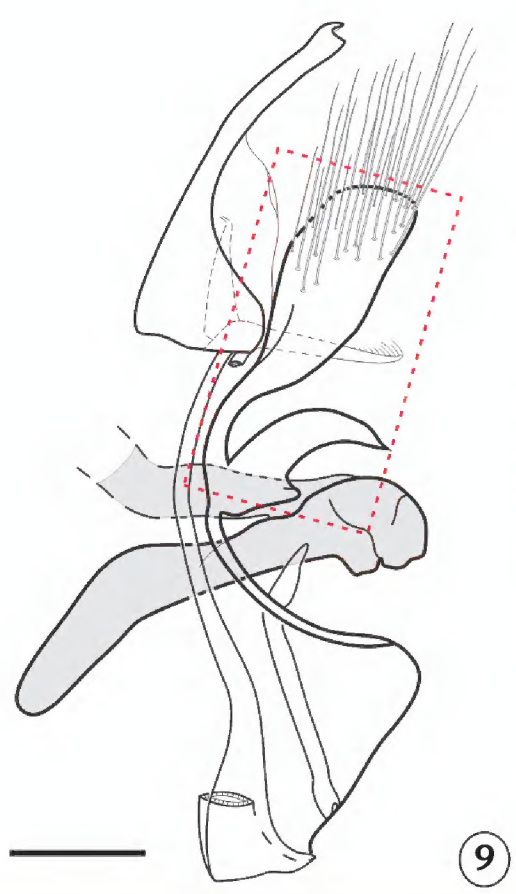
Description of adults (Figs 2–19). **Male.** Forewing length (mean ± standard error) = 9.34±0.45 mm; n = 6. Body with most scales greyish brown, interspersed by either isolated or small patches of either entirely light grey or bicoulored scales, mostly with whitish beige at the base and pale greyish brown at the apex (Fig. 2). Palpi and thoracic legs a little lighter at distal portion of each segment (Figs 7, 8). Abdominal segments also lighter ventrally. Head bearing paired tufts of scales on posterior vertex that project mesally, forming a collar (Fig. 6). Similar to description of *P. crypsicroca* provided by Meyrick (1931): maxillary palpus small; labial palpus well developed, porrected, with middle segment more than double the other two in length (Fig. 7); antennae filiform, reaching ca 2/3 forewings in length, with flagellomeres ventrally ciliated; forewings mostly dark grey; a wavy whitish line on costal edge marking alternate dark fuscous spaces; a short and fine whitish, transversal bar on end of cell; three wavy whitish parallel lines crossing the terminal lobes (Fig. 2), proximal one weakly defined, restricted to basal

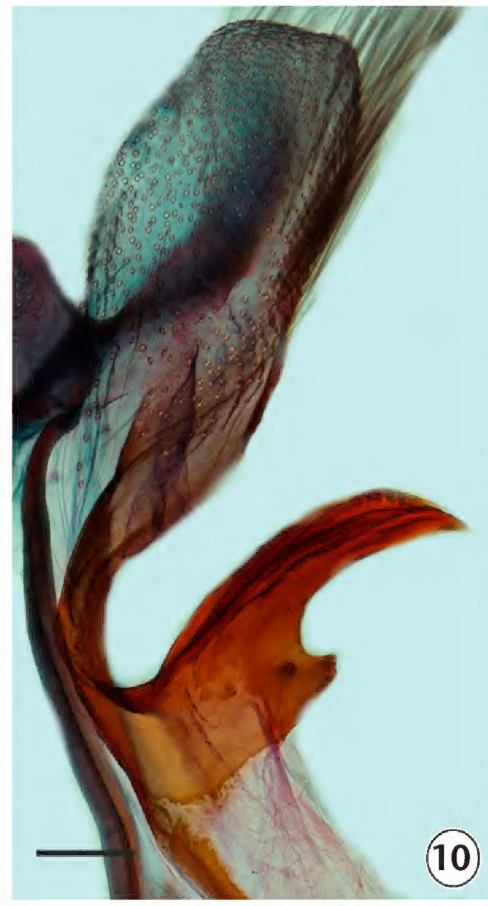


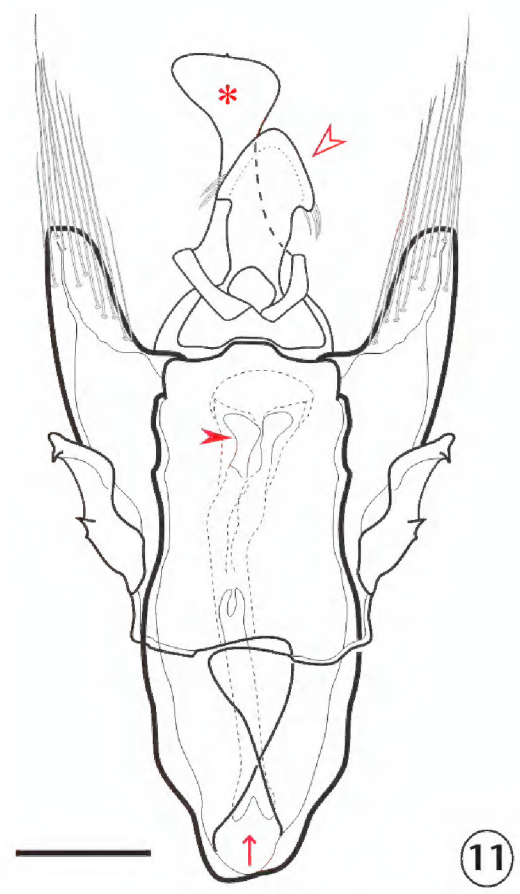


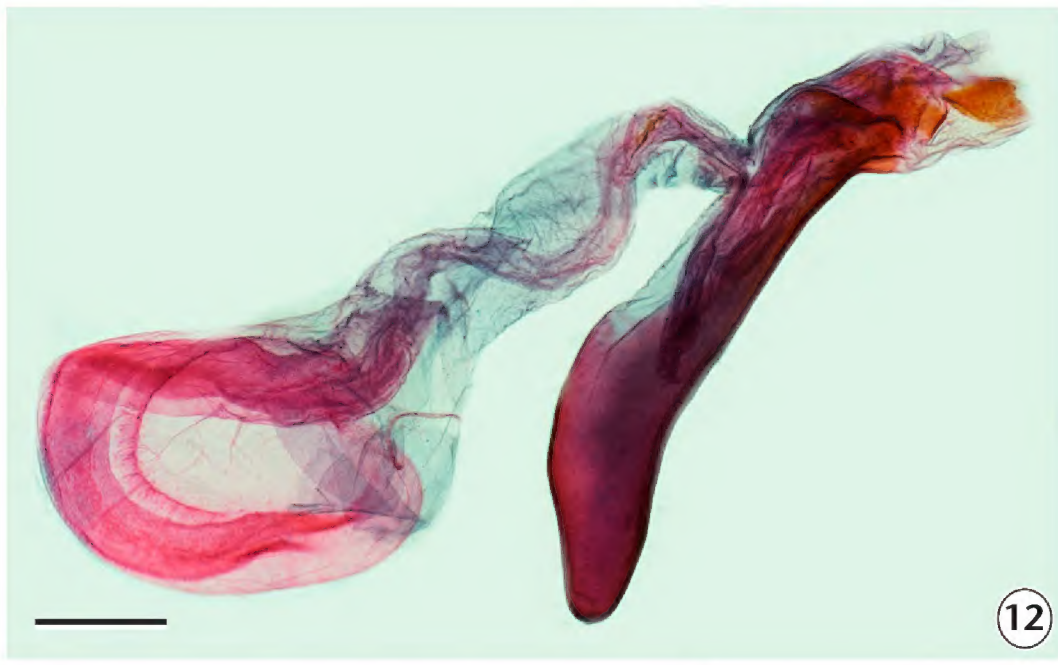


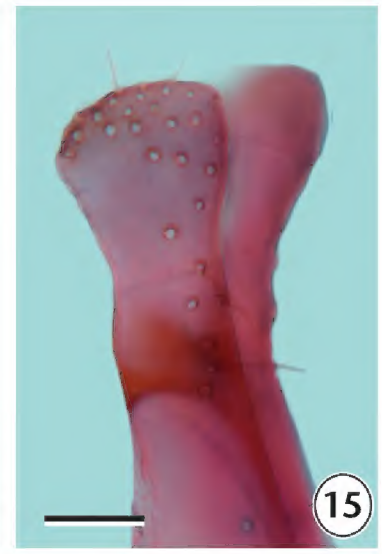
Figures 2–8. Pinned-dried adult of *Prymnotomis cecidicola* sp. nov. (2) dorsal view, and corresponding morphology (3–7) in detail: (3) right forewing apical angle (indicated by rectangle in Fig. 2); (4) left hind wing discal cell area (marked by asterisk in Fig. 2); (5) right hind wing outer margin (delimited by brackets in Fig. 2); (6,7) head in dorsal and lateral views, respectively; (8) left hind leg, anterior view. Scale bars: 2.5, 1, 1, 1, 0.5, 0.5, 2 mm, respectively.



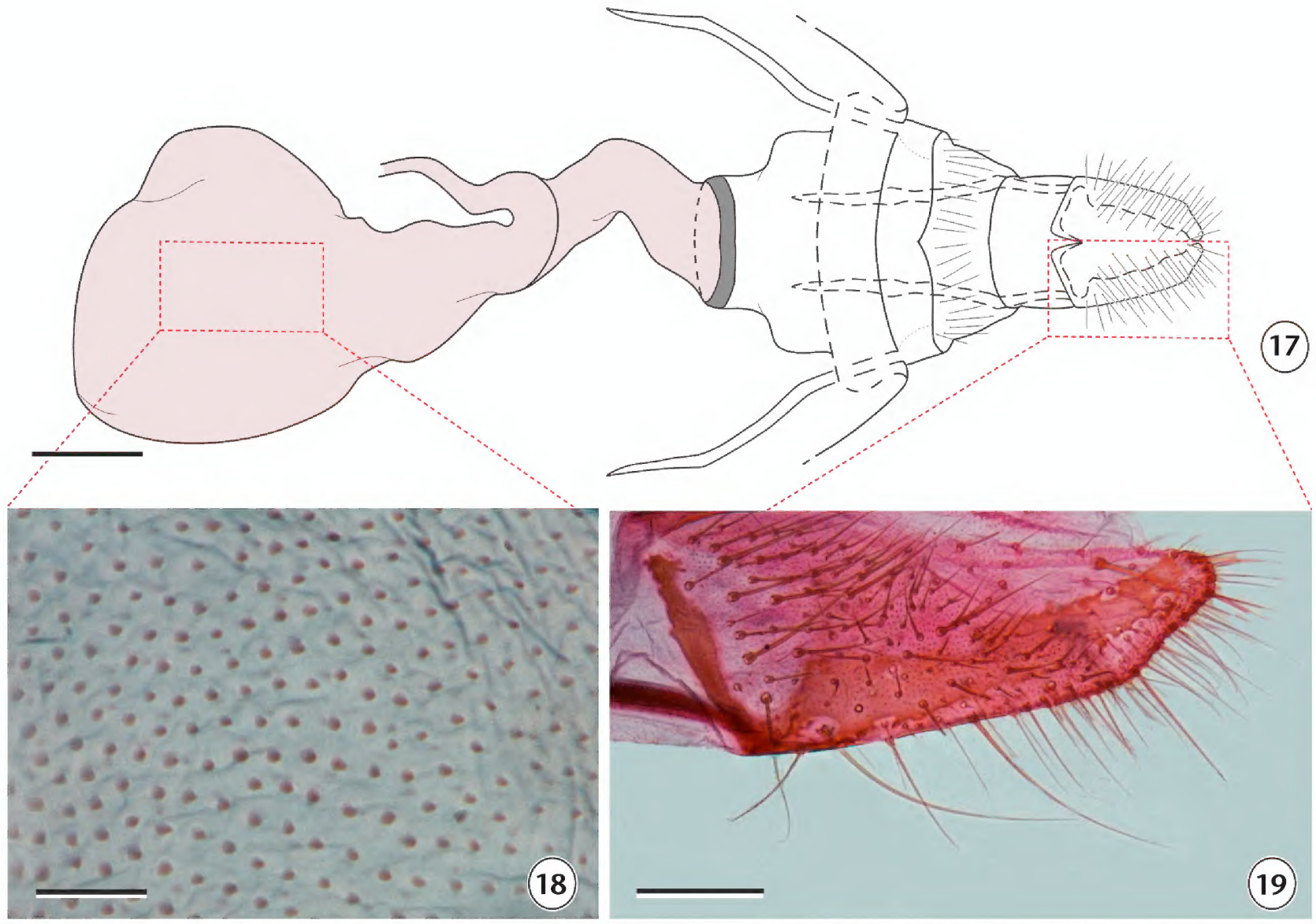








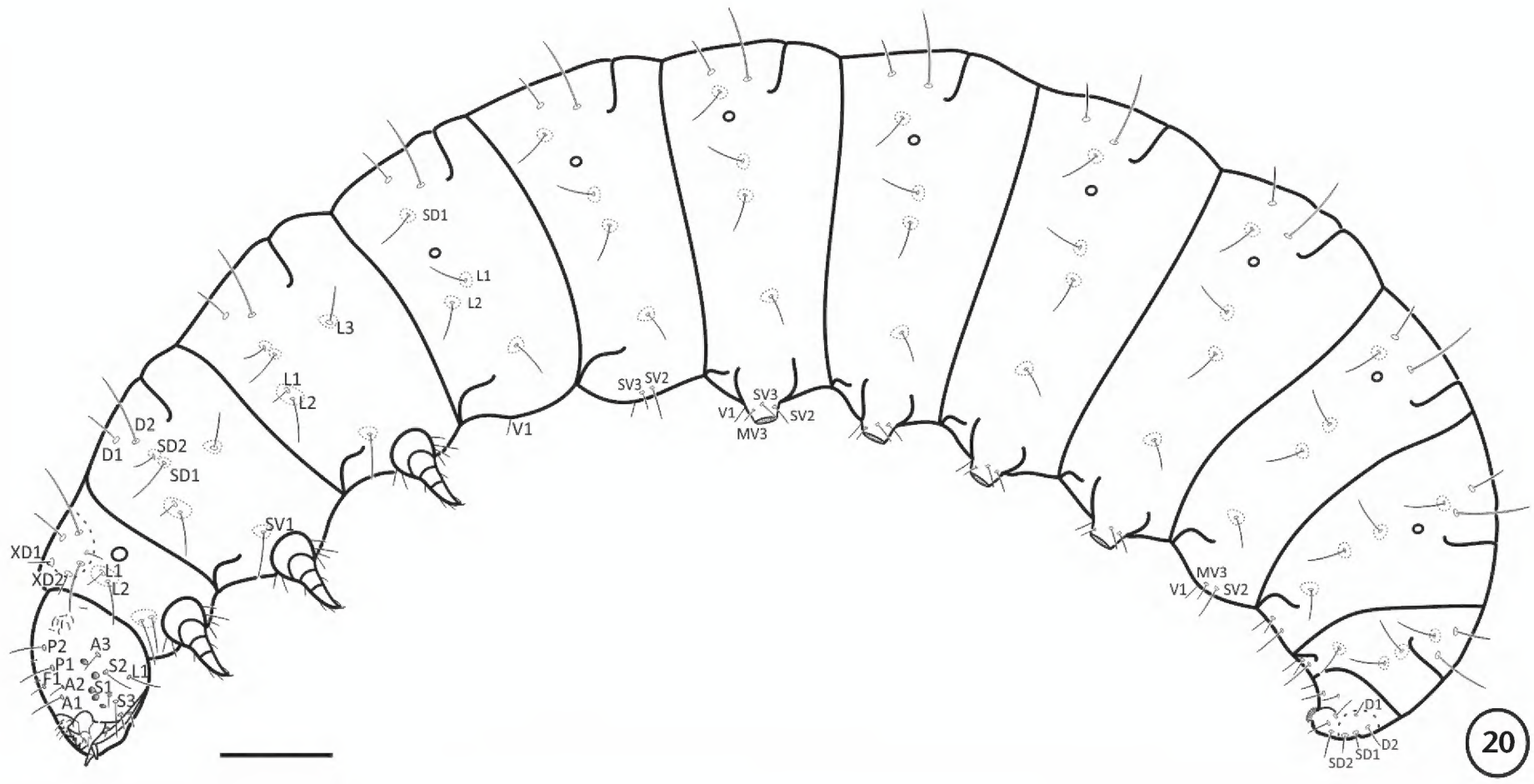
Figures 9–16. Male genitalia morphology of *Prymnotomis cecidicola* sp. nov. under light microscopy: (9, 11) general, lateral and ventral views, respectively (aedeagus omitted in Fig. 11); (10) valva, lateral (area marked with rectangle in Fig. 9); (12) aedeagus, lateral; (13) apex of uncus, ventral (indicated by asterisk in Fig. 11); (14) distal portion of gnathos (pointed by open arrow in Fig. 11); (15) arms of juxta, lateral (indicated by closed arrow in Fig. 11); (16) base of juxta (pointed by seta in Fig. 11). Scale bars; 200, 100, 200, 200, 100, 100, 70, 100 µm, respectively.



Figures 17–19. Female genitalia morphology of *Prymnotomis cecidicola* sp. nov. under light microscopy: (17) general view, ventral; (18, 19) detail of corpus bursae and papillae anales, respectively (areas marked with rectangles in Fig. 17). Scale bars: 200, 30, 50 µm, respectively.

intersections (Fig. 3); fringe concolours with adjacent scales; hind wings mostly dark grey distally, with basal half whitish, densely permeated on base with dark grey scales (Fig. 4); three fine white wavy lines on lobes and associated fringes (Fig. 5), similar to description of forewings. Genitalia (Figs 9–16): Uncus narrow, downcurved near middle and spatulate (Figs 9, 11, 13), with distal margin showing medially a slightly developed, pointed process. Median arm of gnathos strait and flattened, with a bow-shaped, distal margin bearing filiform setae (Figs 9, 11, 14). Tegumen short, compact, rounded dorsally (Fig. 9). Juxta straight, narrow and flat, forked on both ends (Figs 11, 16) with a pair of long, cylindrical arms bearing minute and sparse setae on widened distal ends (Figs 11, 15). Valva membranous, with cucullus well developed, widened in distal half, bearing long filiform setae on distal, rounded margin; basal process well developed and sclerotized, claw-like, bearing a few stout spines on base (Figs 9–11). Aedeagus similar in length to valva, tubiform, slightly curved, bearing a pair of indistinctly shaped, sclerotized plates apically. Vesica with elongated and narrow plates of indistinct shape. Coecum penis ca two-thirds of total length of aedeagus (Figs 9, 12). **Female.** Virtually no differences from male regarding size and coloration. Genitalia (Figs 17–19): Papillae anales elongated, narrowly rounded apically, bearing sparse, long and short setae (Fig. 19). Eighth tergum broad with relatively long setae at distal on ventral margin. Posterior and anterior apophyses thin, similar to each other in length. Ostium bursae broad, opening near posterior margin of eighth sternum; antrum narrower, wide medially, slightly sclerotized on distal margin; ductus bursae membranous, wide medially, similar to anterior apophyses in length. Ductus seminalis inserted on distal third of ductus bursae. Corpus bursae membranous, ovoid, ca 2/3 ductus bursae in length, wall covered with minute microtrichea (Fig. 18), without signum.

Description of immature stages. **Last instar larva** (Figs 20–34, 51): Head capsule width (mean ± standard error) = 1.88±0.01 mm; body length = 15.24±1.31 mm, n = 5. Body slightly cuneiform, proportionally wider in the middle abdominal segments (Fig. 20). Head tan-brown, with frontoclypeal area, labrum and mandibles darker (Figs 21–23, 51). Thorax and abdomen light yellow (Fig. 51), turning into reddish prior to pupation. Prothoracic shield slightly melanized, with faint patches of light brown spots located laterally. Anal plate and thoracic legs not melanized. **Head**: subrectangular, with lateral margins convex, semiprognathus; a sculptured area latero-dorsally on posterior margin (Figs 24, 27). Frontoclypeus subtriangular, with adfrontal sutures extending to apex of epicranial notch (Fig. 21).



Figures 20–23. Last larval instar of *Prymnotomis cecidicola* sp. nov. under light microscopy: (20) chaetotaxy, lateral view; (21–23) head under dorsal, ventral and lateral views, respectively. Scale bars: 1 mm; 100, 100, 100 µm, respectively.

Five poorly developed, laterally located stemmata (Figs 22, 24). Labrum (Fig. 25) slightly bilobed, with three pairs of setae on distal margin, two pairs laterally on proximal margin and another pair mesally. Antenna (Fig. 26) 2-segmented; basal segment with five sensilla on distal margin, two short and stout, one minute, and two long with more than 3x the length of the others; distal segment much thinner and shorter, bearing one short sensilla on distal margin. Mandible well developed with four cusps along distal margin and two setae basally on external surface. Maxilla (Fig. 28) with palpus and galea well developed, stipes bearing well-developed flap-like, distally forked protrusions that project mesally (Fig. 29). Spinneret short, conical (Figs 28, 29). Labial palpus (Fig. 29) bi-segmented; distal segment thinner and much shorter, both with well-developed apical seta. Chaetotaxy (Fig. 20): MD group trisetose; F unisetose; C group bisetose; A group trisetose, with A1 longer than A2 and A3; AF group bisetose; S group trisetose, with S2 and S3 longer than S1; L unisetose, SS group trisetose. **Thorax (T) and abdomen (A)**: Integument covered with microtrichia (Figs 30, 33, 34). Thoracic legs well developed (Fig. 30), with stout tarsal claw bearing a tooth on ventral basis (Fig. 31). Circular spiracles (Fig. 32) with slightly elevated peritreme, laterally on T1, and A1–8. Abdominal pseudopodia short (Figs 33, 34), on A3-6 and A10, with crochets arranged on uniserial and uniordinal, as a penellipse. Chaetotaxy (Fig. 20): T1 with D, XD and SD group bisetose, all on the dorsal shield; D2 longer than D1; XD similar to each other in length; SD2 shorter than SD1; L group bisetose, both on the same pinacula, with L1 shorter and latero-dorsal to L2; SV bisetose, with SV1 shorter than SV2. T2-3 with D group bisetose, similar to prothorax; SD bisetose; L1 trisetose, with L1-2 anterior to L3, and posterior

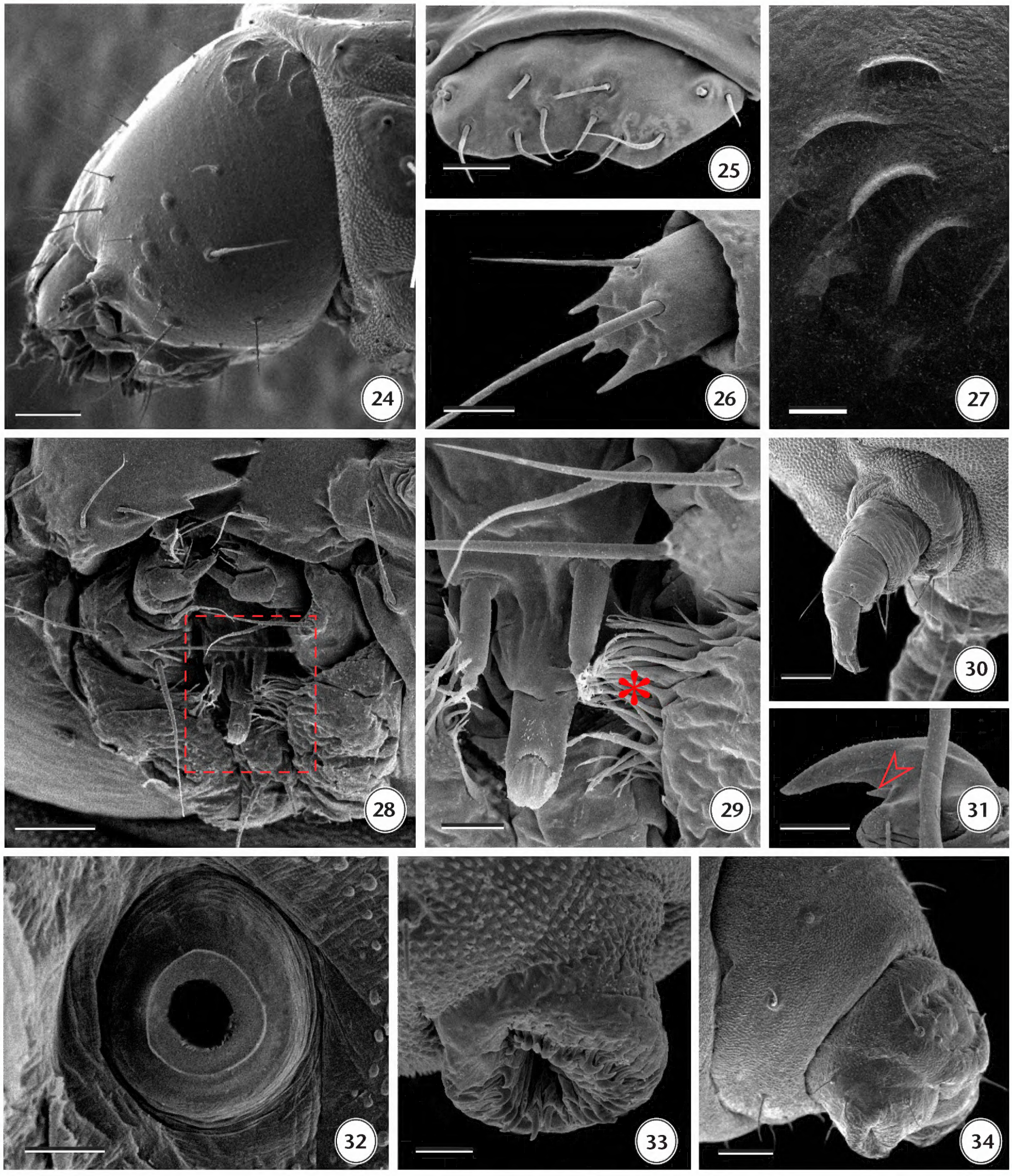
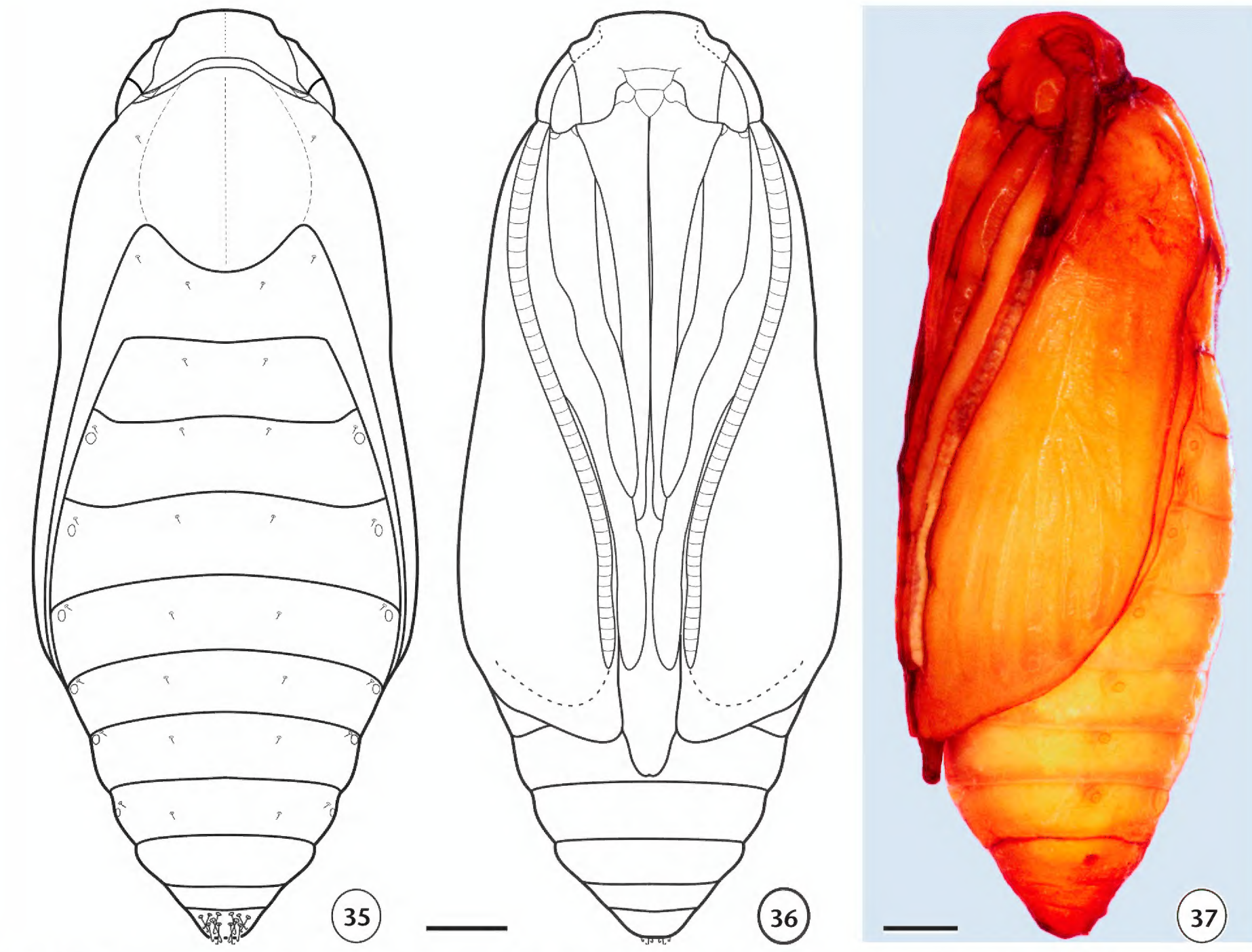


Figure 24–34. Morphology of *Prymnotomis cecidicola* sp. nov. last larval instar under scanning electron microscopy: (24) head, lateral view; (25) labrum, dorsal; (26) antenna, lateral; (27) latero-dorsal area of head in detail; (28) maxillae and labium, antero-ventral; (29) labium in detail (area marked by rectangle in Fig. 28; asterisk indicates associated flap-like protrusions of stipes); (30) mesothoracic leg, postero-lateral; (31) tarsal claw in detail, posterior (open arrow indicates basal spine); (32) prothoracic spiracle, lateral; (33) proleg of fifth abdominal segment, lateral; (34) last two abdominal segments, lateral. Scale bars: 200, 50, 50, 50, 100, 25, 100, 25, 50, 50, 200 µm; 0.5, 0.5, 0.5 mm, respectively.

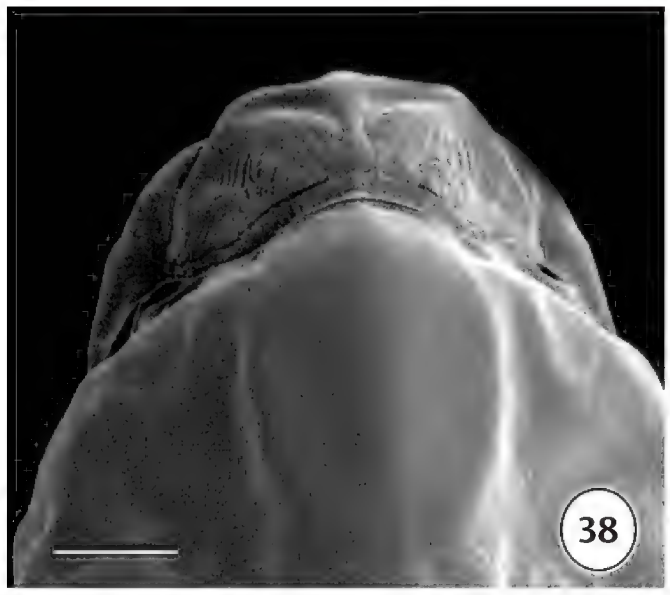
to SD; SV and V unisetose. Abdominal segments (A) with only short setae that are more or less aligned on the middle region of each segment, which are herein tentatively named. A1 with D and L groups bisetose; SD and SV unisetose; V present. A2 with D and L groups bisetose; SV trisetose; SD unisetose; V present. A3-6 with D and L groups bisetose; SD unisetose; SV trisetose, with SV2 and SV3 on proleg; MV3 and V present. A7-9 with D, SV and L groups bisetose; SD unisetose; MV3 and V present. A10 with D and SD groups bisetose, located within the anal plate; L bisetose; SV trisetose; V present.

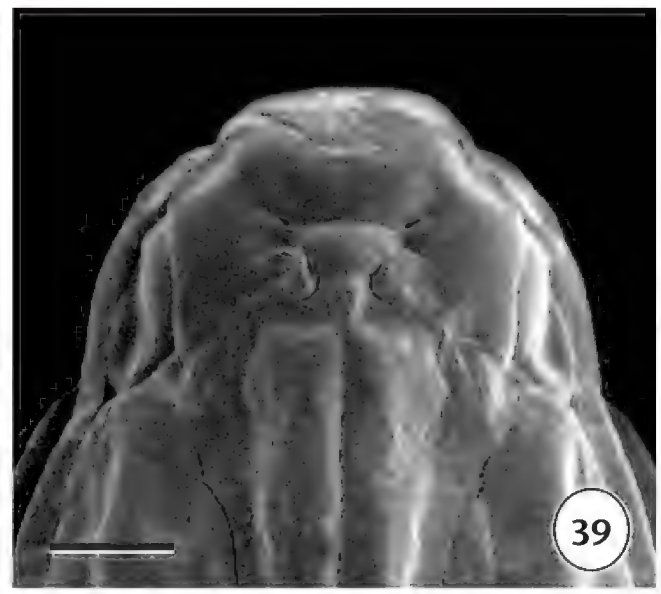
Pupa (Figs 35–48). Body cylindrical, yellowish brown, mean length (± standard error) = 9.6 ± 1.74 mm; maximum width = 4.08 ± 0.07 mm; n = 5. Head with vertex deprived of setae and without a differentiated gall-cutter (Figs 38–41). Frons wide, posteriorly expanded mesally to the eyes. Clypeus subtrapezoidal, also without setae. Antennae filiform, reaching the distal end of the middle legs. Mandibles small, rounded, latero-posterior to the clypeus. Maxillary palpi small, rounded, latero-posterior to the eyes. Proboscis well developed, reaching A2. Prothorax fairly developed, deprived of setae, bearing postero-laterally a wide open spiracle (Fig. 43). Hindwings concealed by forewings; the latter with six well marked longitudinal lobes on external surface (Fig. 44), extending to sixth abdominal segment. Protho-, meso- and methatoracic legs reaching the third, fifth, and seventh abdominal segments, respectively. Thoracic and abdominal setae extremely reduced in size (Fig. 45); one pair dorsally on mesothorax and A1; two pairs of such setae on metathorax and A2-7, one dorsal and the other lateral, dorsally to spiracles. Abdominal spiracles rounded, with slightly elevated peritreme (Fig. 46), laterally on A2–7; spiracle on A8 partially closed (Fig. 47). Distal margin of the last abdominal segment with six pairs of stout, distally hooked setae (Fig. 48).

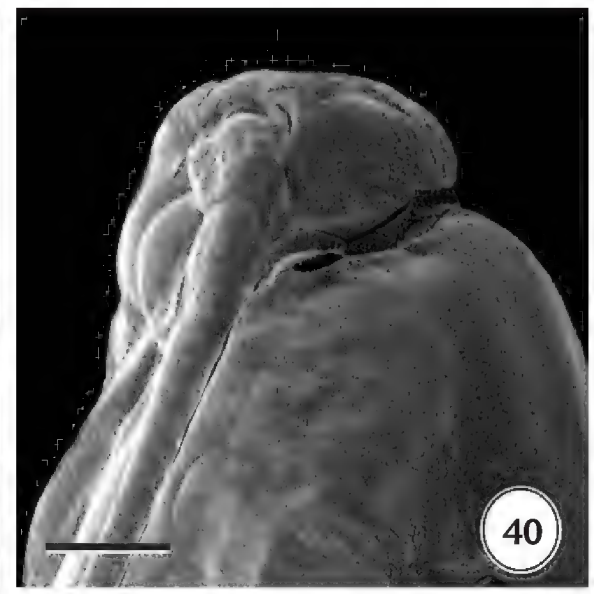
Material examined. All specimens examined came from galls associated with *Cordiera elliptica* (Cham.) Kuntze (Rubiaceae) at Embrapa Cerrados, as already described. Adults were reared by V.O. Becker, from galls collected during October 1982-83

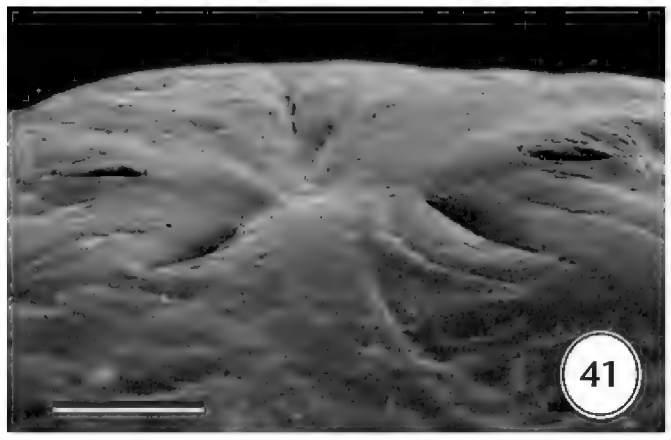


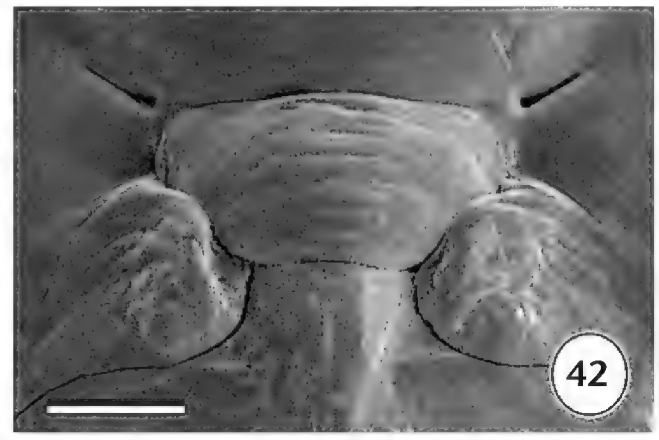
Figures 35–37. *Prymnotomis cecidicola* sp. nov. pupa under light microscopy, in dorsal (35), ventral (36) and lateral (37) views. Scale bar: 0.5 mm.

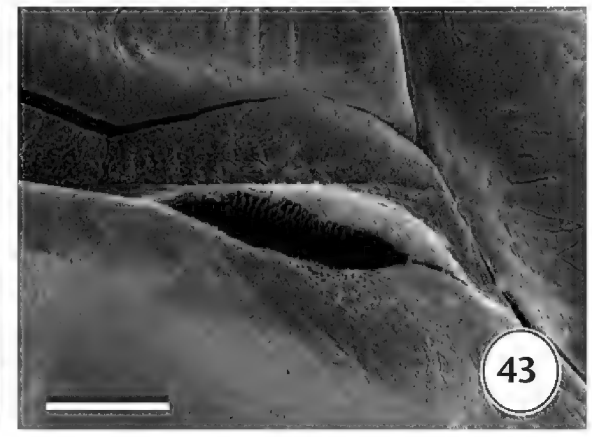


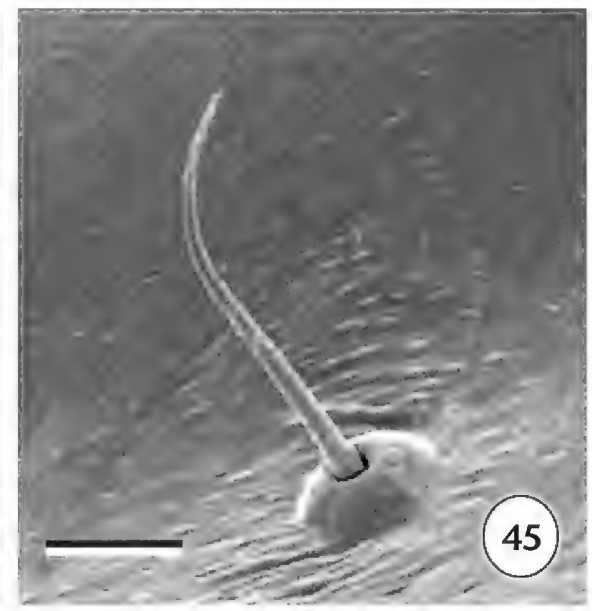


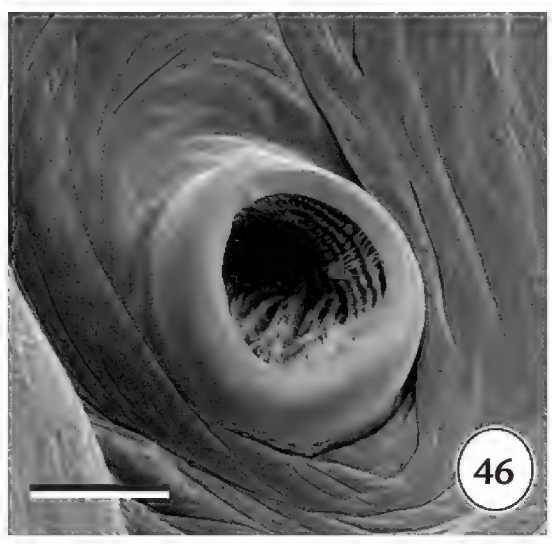


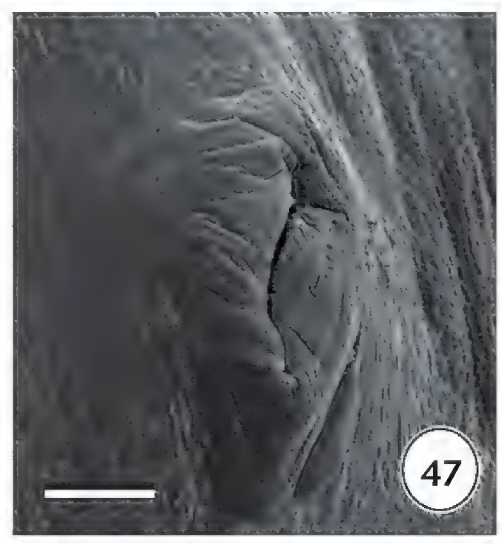












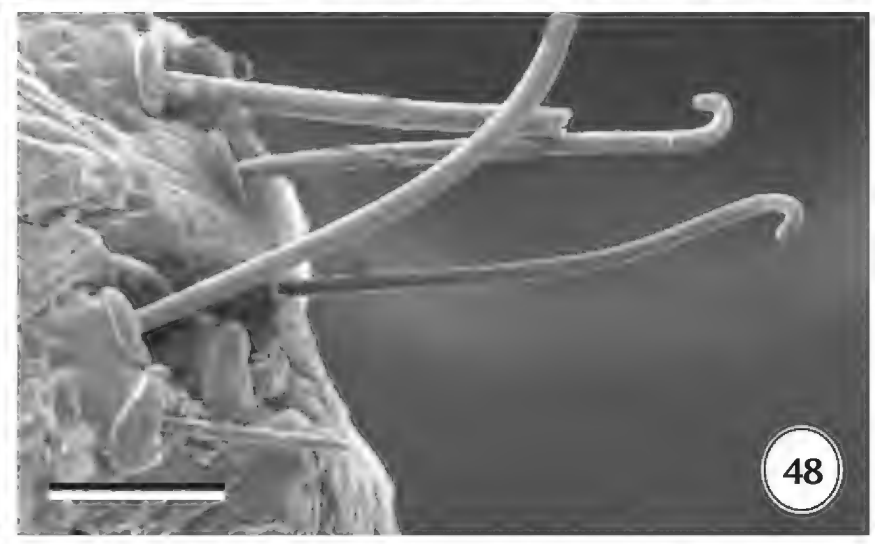


Figure 38–48. Morphology of *Prymnotomis cecidicola* sp. nov. pupa under scanning electron microscopy: (38–40) head, under dorsal, ventral and lateral views, respectively; (41) vertex of head, anterior; (42) buccal appendages, ventral; (43) prothoracic spiracle, dorsal; (44) left forewing, lateral; (45) mesothoracic seta, dorsal; (46, 47) fourth and eight abdominal spiracles, respectively, lateral; (48) dorsal hooks of last abdominal segment, lateral. Scale bars: 500, 100, 200, 100, 500, 25, 50, 50, 50 µm, respectively.

(LMCI 313 series). Immatures were either dissected or reared by C.M. Pereira and A. Specht, from galls collected by C.M. Pereira (LMCI 349 series) on 19.x.2018. Additional galls were collected by G.R.P. Moreira & J. Fochezato (LMCI 346 series) on 1-4.xii.2018.

Type material. Holotype male, BRAZIL: DF, Planaltina, 1100 m, 14.i.1985, ex *Cordiera elliptica*(Cham.) Kuntze (Rubiaceae) (Becker, 57100) (VOB). Paratypes: 11 males, 4 females, 3.xii.1984-8.i.1985, same data as holotype; 1 female, same data as holotye, but 20.ii.1976, at light (Becker, 19564); 2 male, 1 female, same data as holotype, but 15-30.xii.1982 (Becker, 40740); 1 male, same data as holotype, but 15.x.1982, at light (Becker, 40613); 11 males, 1 female, same data as holotype, but 14.xi-23.xii.1983, at light (Becker, 41731); 1 male, same data as holotype, but 5.i.1984, at light (Becker, 56053). Additional paratypes, same data as holotype: 3 male (VOB 57100, 3.xii.1984, deposited LMCI 313-869; VOB 41731, 26.xii.1983, deposited at LMCI 313-874; LMCI 313-879/VOB 40740, donated to DZUP/DZ 33.402), and 3 ♀♀ (VOB 57100, 19.xii.1984, deposited LMCI 313-870; VOB 41731, 5.xii.1983, deposited at LMCI 313-876; VOB 41731, 14.xii.1983, donated to DZUP/DZ 33.412).

Additional material examined, not type specimens: 1 female, GO, Ipameri, 10.X.1988 (Becker, 59682); MG, Nova Lima, 850m: 2 males, 25-27.xii.1982; 2 males, 1-10.i.1985; 1 female, 30.xii.1988; all at light (Becker, 50290, 55772, 60523) (VOB, USNM, NHMUK). Pinned-dried adults with genitalia preparations mounted in Canada balsam on slides – 2 males (VOB 40740, 17.i.1983, deposited at LMCI 313-867; VOB 40740, 15.xii.1982, deposited at LMCI 313-880); 2 females (VOB 57100, 6.xii.1984, deposited at LMCI 313-872; VOB 40740, 30.xii.1982, deposited at LMCI 313-878). Immatures fixed in Dietrich's fluid and preserved in 70% ethanol – three last instar larvae (VOB 1519, 2.x.1984, deposited at LMCI 313-865A); five pupae (VOB 1519, 2.x.1984, deposited at LMCI 313-865B); twelve mature galls (CMP 008-01, donated LMCI 349-1); five empty, senescent galls (LMCI 346-01). Also, two last instar larvae, preserved in 100% ethanol at -20 °C, used for DNA extraction (CMP 008-04, donated to LMCI 349-2). Two last instar larvae preparations, also mounted in Canada balsam on a slide (VOB 1519, donated to LMCI 313-865C).

Other species examined (pinned-mounted adults): *Prymnotomis crypsicroca* Holotype male, BRAZIL: ES, [Baixo] Guandú, ES, 1920 (Hoffmann) (NM, Vienna) (g.s. NM 13322). *Hexeretmis pontopora* Meyrick, 1??? Holotype males, BRAZIL: PA, Taperinha, 11-20.vi.1927 (Zerny) (NM, Vienna) (g.s. NM 13321. *Alinguata neblina* Fleming, Alotype male, VENEZUELA: [Aragua], Rancho Grande, 3.vii.1946 (*Fleming*) (AMNH) (g.s. VOB).

Distribution. *Prymnotomis cecidicola* is known from the Brazilian Savanna, within the biome called "Cerradão" (= Cerrado *stricto sensu*; for a description, see Parron et al. 1998) where their host-plant and associated galls were found. The adults of this species do not come readily to light, as shown by the few specimens studied. The third author collected all over the Cerrado region of Brazil for over 30 years. At the type locality (Planaltina) he collected this species regularly in places very close to the host plant where the galls were common.

Host plant. Galls of *P. cecidicola* have been found only in association with *Cordiera elliptica* (Cham.) Kuntze (Rubiaceae) (Fig. 49), a plant native to the Brazilian Savanna (Cerrado Biome), with distribution ranging from northeast Bahia to southeast São Paulo state. It is a dioecious shrub (1.5 to 3.0 m high), with thin, cylindrical, glabrous branches, having dimorphic inflorescences; these are fasciculate on male plants, but only solitary flowers appear on female ones (Matsuoka LG, UFG, unpublished data). Also according to this author, flowers appear during the dry season (from July to September), fruits maturing later on, during the peak of the rainy season (from November to December), which was confirmed by our field observations. At the type locality, *C. elliptica* is commonly known as "marmelinho" and "marmelada-de-pinto", being found scattered on vegetation, particularly along trails. The fruits are consumed by native people either fresh or as a home-made jelly.

Etymology. The species name is derived from the Greek *kekis – idos* = gall + the Latin *co – col* = with; to be treated as feminine.

Natural history. Mature galls of *P. cecidicola* (Figs 50–53) measure on average (± standard error) 1.80 ± 0.34 cm (n = 5) in diameter. They are green when active, spherical, unilocular and develop individually on *C. elliptica* inflorescences of both male and female plants. Thus we infer they are induced early on flower buds, since male flowers are dehiscent. *Cordiera elliptica* fruits are also green and spherical during development, but a little smaller (maximum diameter = 1.49±0.14 cm; n = 5) and turn brown when mature (Fig. 55). Contrary to *P. cecidicola* galls that have a smooth surface, *C. elliptica* fruits show conspicuous style scars distally (Fig. 50). Empty galls of *P. cecidicola* dry up, turning black, remaining attached to the plant (Fig. 56).

Larvae of *P. cecidicola* assume an arched position within their galls by placing the body around the fecal pellets left inside the gall. These are packaged and positioned centrally, as a sphere, firmly attached to the gall wall (Fig. 51), except when leaving the gall, when fecal pellets are left aside (Fig. 53). This sphere also contains the larval exuviae packaged within the feces. It is apparently increased in size and modelled periodically, being covered each time by a fine, blackish, silk net.

The body of full-grown *P. cecidicola* larvae progressively changes to red before pupation, when they leave the gall through a circular orifice made laterally on the gall wall (Fig. 52). Invariably when offered sandy soil and dried-broken leaves at the bottom of the rearing plastic pots in the laboratory, they built a flat, semi-rectangular, tied, silk-woven cocoon with debris attached (Fig. 54). Exit orifices were present on the surface of empty galls found in the field. Since we did not encounter cocoons attached to the host plant bearing empty galls under these conditions, we presume pupation occurs in the litter, which should be explored further.

Figure 49–56. Natural history of *Prymnotomis cecidicola* sp. nov. on *C. elliptica*: (49) host plant at the type locality; (50) young fruit and gall on female plant (gall is marked by asterisk; closed arrow indicates style scar of the fruit); (51) dissected gall showing last instar larva (seta indicates sphere made of faeces and exuviae attached to the gall wall); (52) external aspect of empty gall, showing larval exit orifice (pointed by open arrow); (53) dissected empty gall, showing sphere of packaged faeces and exuviae with fecal pellets left aside (pointed by closed arrow) by the larva before leaving for pupation; (54) fresh cocoon made by last larval instar in association with sand grains and dead-broken leaves under laboratory conditions; (55) mature fruit; (56) senescent empty gall. Scale bars: 3, 3.5, 4, 1, 3.5, 3.5 mm, respectively.

Cordiera elliptica plants bearing galls of *P. cecidicola* were found scattered in the field, density varying from one to four galls per plant. Field collections suggested that at the type locality *P. cecidicola* is a univoltine species. Galls and associated larvae were noticeable during the end of the dry season (August) up to beginning of the rainy season (October), thus coinciding in phenology with the reproductive phase of the host plant, described above. Full-grown larvae and pupae were obtained in late October. Emergence of adults under laboratory conditions was recorded from November to January.

DISCUSSION

This study sheds light on the biology of *Prymnotomis*, a poorly known genus of Neotropical many-plumed moth. Lack of morphological and molecular data on the other three alucitid genera endemic to the region makes a broad discussion about the descriptions presented here difficult. However, field collections in Costa Rica, Puerto Rico and French Guiana that resulted in several barcoded specimens (clustered in five BINs) allowed us to compare *Prymnotomis* with other Neotropical material from a genetic perspective. As expected, our sequence clustered with these BINs and was most closely related to cluster #5, presenting ca 12% genetic distance. Thus a revision is needed in Alucitidae, also to reduce the gap of lineage coverage in the analysis, which likely influences the large genetic divergence between taxa. The most distant affinity was found between *Prymnotomis* and *Pterotopteryx*, a Paleartic genus, not represented in the Neotropics, and that contrary to *Prymnotomis* shows deeply divided wings. *Prymnotomis* is expected to be more closely related morphologically to *Hexeretmis* and *Paelia*, according to Meyrick (1931). This should be further explored, also taking into account morphological characteristics of immature stages, their host plants and larval feeding habitats not only for these genera but also including *Alinguata*, also endemic to the Neotropics. Their original descriptions were based mostly on coloration and relative depth of wing lobes.

The unusual flap-like protrusion on stipes described here for the larva of *P. cecidicola* has been found in other alucitds, and also in the closely related copromorphid and carposinid lineages (Heppner 1987). We confirmed the suggestion of this author that these structures are functionally associated with the spinneret. As described by him, the tip of the spinneret is nested within these flaps in *P. cecidicola*. Additional observation demonstrated that they are used to retain partially a brownish dark, liquid substance over the spinneret's tip, which is used to soak the silk strands continuously when they emerge during the weaving process. This substance then solidifies, sealing the silk threads of the net that is used, for example, to cover the faecal pellet described here, and that thus remains isolated from the larva within the gall. We also noted under laboratory conditions that an orifice artificially made on the gall wall is immediately covered by the larva in this manner. Whether this substance is regurgitated and/or produced by an exocrine gland associated with its buccal apparatus should be further explored.

The absence of a differentiated cocoon-cutter and abdominal spines on the pupa of *P. cecidicola* and the presence of curved-pointed hooks on the terminal portion of the abdomen suggest that adult emergence in this species occurs inside the cocoon, which should be further examined. The emergence of the adult on the pupation site apparently appeared earlier in Lepidoptera evolution, within the Gellechioidea (e.g., Powell 1973, Becker 1982, Luz et al. 2014). Also interesting are the six raised lobes that appear externally on the *P. cecidicola* pupa forewing, particularly under scanning electron microscopy. Further studies should explore whether this characteristic is unique to and how variable it is within the Alucitidae, in order to rank its value as a diagnostic character for the family in this stage. From an ontogenetic perspective, such lobes supposedly correspond to early divisions in the wing that are present on the adults (for a description of the corresponding position related to wing veins in adult alucitids, see Dugdale et al. 1998).

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